BIOCHEMISTRY OF PLANT DISEASES

III. EFFECT OF SCLEROTINIA CINEREA ON PLUMS[1]

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(WITH SEVEN FIGURES)

From the viewpoint that a fungus attacks a host plant, not to destroy it, but to gain a livelihood, it becomes of interest in the study of the chemistry of resistance to discover why a fungus can parasitize some varieties of a host and not others. There may be several bases for this difference: (1) the structure of the resistant host may offer mechanical difficulties to the entrance of the parasite; (2) the host may contain or produce repellent substances, such as tannins, acids, antienzymes, and antibodies; or (3) the host may fail to furnish the proper kinds and amounts of nutrients for the normal development of the fungus.

Each of these possibilities has received some attention at the hands of investigators; but the two latter, constituting what may be called the biochemical basis of resistance and susceptibility, have received the least. It was decided, therefore, to attack the problem of resistance and susceptibility in plants from the standpoint of the nutrition of the parasite, using the brown rot organism of stone fruits, *Sclerotinia cinerea*, as the experimental organism. The first paper in this series dealt with the vitamine requirement of the fungus (**52**), the second with its relations to the pectic substances of the host (**53**); the present paper deals with the composition of certain varieties of plums, and the changes in composition brought about during the process of rotting by the fungus.

Previous work

Cook and Taubenhaus (**16, 17**) found that a great many fungi are very sensitive to tannin, and they believed that this could be a limiting factor in their ability to attack certain plants. In general, parasites are more sensitive to tannin than saprophytes.

[1] Published with the approval of the Director as Paper no. 236, Journal Series, Minnesota Agricultural Experiment Station.

They demonstrated an oxidizing enzyme that produces tannin from gallic acid, and found that the abundance of this enzyme in fruits is correlated with their resistant properties. BASSETT and THOMPSON (3) studied this enzyme still further. KNUDSON (37) found that *Aspergillus* and *Penicillium* can utilize tannin as a source of carbon, by means of the enzyme tannase. That genera and even species within a genus vary greatly in their sensitiveness to tannin was shown by COOK and WILSON (18) in studying the chestnut blight. In the case of *Sclerotinia*, VALLEAU (46) failed to find any correlation between tannin content and resistance in plum varieties.

In recent years considerable attention has been given by investigators to the relation between the H-ion concentration of the soil and that of the plant, and between the latter and its resistance properties. In 1912 COMES (15) announced that the wheats that were more resistant to rust had more acid saps, and that fertilizers which would increase the acidity of the sap would convey added immunity to the plant. Although others have since failed to corroborate these statements, positive correlations have been established between the acidity of the soil and the occurrence of potato scab (23, 24), between the acidity of grape saps and their resistance to disease (2), between the H-ion concentration of soil and that of the plant juice (14, 45), and between spinach mosaic and the PH value (27). On the other hand, a lack of correlation between the acidity of the host and its resistance properties has been found in the case of potatoes toward *Pythium debaryanum* (32), *Phytophthora infestans* (35), and *Chrysophlyctis endobiotica* (50). WAGNER (47, 48) noticed that certain plants increased their acidity when infected with bacterial pathogens. The acidity returned to normal after a brief period, unless the plant were unable to withstand the attack, in which case there took place a sudden fall in acidity much below normal, the death of the tissue, and then a post mortem rise in acidity. The relation of H-ion concentration to the metabolism of fungi and bacteria has received some attention (25, 38, 49). SCHMIDT and HOAGLAND (41) give an extensive bibliography on the relation of bacteria to the reaction of the medium. In general the fungi have been found to be less sensitive than bacteria to the reaction of the medium, and hence fewer

instances are known where the reaction of the sap is a controlling factor in a fungus disease. In the case of *Sclerotinia cinerea* no measurements have been made of its relation to the P_H value of the medium, except some rough titrations made by COOLEY (**19**) on cherry juice, which showed that this fungus can grow and sporulate through a considerable range of acidity and alkalinity.

Many investigators have sought for the mechanism by which fungi penetrate through the tissues of the host plant. Without going into the voluminous literature on this subject, it may be said that several different ways have been found: (1) by mechanical pressure, (2) by enzymes which dissolve either the cell walls or the middle lamellae (**12, 13, 19, 46, 53, 54**), and (3) by toxic substances other than enzymes, especially oxalic acid (**6, 7, 9, 12, 13, 19, 42, 46, 53, 54**). COOLEY found oxalic acid produced by *S. cinerea* in small amounts, and VALLEAU demonstrated that solutions of this acid would disintegrate tissues, probably by removal of calcium from the pectic material of the middle lamella.

The changes in composition which tissues undergo when rotted by organisms, and the differences in composition between resistant and susceptible varieties, have received considerable attention. One of the earliest of such studies was by BEHRENS (**4**), who used a number of fungi, among them *Sclerotinia fructigena*, on apple. REED (**39**) found that *Glomerella* not only decreases the acidity of apples and of synthetic media, but actually makes the latter alkaline. HAWKINS (**28**) found that *Glomerella cingulata* grown on peaches could hydrolyze and assimilate the pentosans, as well as utilize the monosaccharides, but that *S. cinerea* could not utilize the pentosans of the peach (**29**). In the latter case there was an increase in titratable acidity during the rotting. In the case of potato tubers infected with various species of *Fusarium* (**31**) there was a decrease in sucrose, reducing sugars, pentosans, galactans, and dry matter; an increase in crude fiber, due to its formation in the hyphae of the fungi; and no change in the starch and methyl pentosans. BISBY (**6**) and EDSON (**21**) also reported no effect on the starch of potato tubers by certain fungi, and suggest that potato starch could be made from rotted tubers. VALLEAU (**46**) examined many plum varieties as to their content of tannin, but

could find no correlation between this factor and their resistance to brown rot. CULPEPPER, FOSTER, and CALDWELL (20) made detailed analyses of apples infested with *Sphaeropsis malorum*, and found that the rotted fruit had undergone considerable loss in dry matter, a loss in alcohol-ether-water-alcohol extractives, an increase in protein nitrogen and in protein phosphorus, a transfer of minerals from the insoluble to the soluble fraction, a loss in total sugars, mostly in the monosaccharides, a decrease in titratable acidity, and a marked increase in alcohol. Starch was not affected.

STEVENS and HAWKINS (43) adopted a procedure that elucidates the progressive changes during rotting, by analyzing (1) the fresh strawberry fruits, (2) the sound fruit after storage under the same condition as the inoculated fruit, and (3) the fruit inoculated and rotted by *Rhizopus nigricans*. These three samples show the parallel changes in sound and infected fruit. They found that the acids in the sound fruits decreased, probably due to respiration, and that the acids decreased to a less extent in the rotted fruit. The authors believed this to be due to an interference with the tissue respiration by the fungus and not to the production of ammonia. Sucrose, reducing sugars, and dry matter decreased more rapidly in infected than in sound fruit. The fungus causes the tissue to soften and to become watery, but whether this is due to the death of the cells or to an anesthetic effect is still an open question. STEVENS and MORSE (44) reported that in the end rot of cranberries there is a marked decrease in sugars, while the proximate constituents remain fairly constant. The protein, fiber, and ash, however, show such relative increases as would be expected from the loss of dry matter by respiration. GIDDINGS (22) and RUSSELL (40) reported the inauguration of studies on apple leaves and on potato tubers, respectively, to determine the chemical basis of resistance, but they offer no conclusions as yet.

Recently several papers have appeared which deal with the nitrogen distribution of diseased plant tissues, and which promise to furnish a new line of attack on these problems. BONCQUET (10, 11), working with the mosaic disease of tobacco, *Streptococcus solani* on potato, and *B. morulans* on beet leaves, reported that nitrites and ammonia were invariably found in diseased but never

in healthy parts. The amount of nitrites was proportional to the intensity of the pathologic condition. The reducing organisms were mostly in the vascular tissues. The reduction of the nitrates brought about nitrogen starvation, with a consequent yellowing and distortion of the affected tissue. In a field where potatoes had been grown continuously for fifteen years, nearly every vine was affected with nitrogen starvation, although the soil contained an abundance of nitrates. In tobacco mosaic it was observable that the plants tended to oppose these chemical forces both by morphological and by physiological means; thus the secondary organs were reduced in size, more water was transpired, and the oxidizing enzymes showed greater activity.

JODIDI, MOULTON, and MARKLEY (34) made a detailed dissection of the nitrogen constituents of spinach mosaic, and found evidences of denitrification, due to the production of nitrites and their subsequent action on amino nitrogen groups. In cabbage mosaic (33) a similar condition was found, hence nitrogen starvation is believed to be the cause of the abnormal appearance of the leaves in these diseases. It is to be regretted that BONCQUET and BONCQUET give none of their methods of analysis, nor any data whatsoever in their papers.

Material

Five varieties of plums, grown at the University Fruit Breeding Farm at Excelsior in 1920,[2] were selected for the work. Three of them show marked resistance to the attacks of the brown rot fungus, while the other two are very susceptible. Samples were picked at three stages of growth: (1) when half grown, (2) when fully grown and just beginning to ripen, and (3) when fully ripe, but still on the tree. In most cases each sample was divided into three portions. One portion was analyzed immediately, another was inoculated with a pure strain of *Sclerotinia cinerea* and placed in a moist chamber to rot, and a third portion was placed in a moist chamber without inoculating and left for the same length of time as the corresponding inoculated portion. The inoculations were made by injecting a suspension of spores with a hypodermic syringe into the

[2] Acknowledgments are due to Dr. M. J. DORSEY for assistance in obtaining the material.

tissues of the plums, after the latter had been sterilized with mercuric chloride. The plums were left to rot as long a time as was practicable, which was usually from five to seven days after all the tissue had turned brown to the stone. The same degree of rotting was not obtained in all cases, since this cannot readily be judged.

Methods

PREPARATION FOR ANALYSIS.—In preparing the samples for analysis the stones were removed, the pulps frozen in an ice and salt mixture for three hours, ground in a food grinder, and pressed in a hydraulic press. All manipulations were maintained as uniform as possible throughout the series. The expressed juice was then used for all the subsequent analyses. HARVEY (**26**) has shown that, in order to obtain the true P_H of a juice, the latter should be expressed without freezing, since the freezing precipitates certain proteins and thus changes the H-ion concentration. This fact had to be ignored in the present instance, however, since the determination of the other solutes must be made on juice from frozen tissue, and since the amount of material available was not large enough to admit of two samples of juice being taken in each case. It might be of interest to record the results of a single test of the effect of freezing. The material was some seedling plums about one-third grown.

	Frozen	Unfrozen
Percentage of pulp obtained as juice	70	60
P_H	1.67	1.48

SPECIFIC GRAVITY.—The specific gravity was obtained by means of a Westphal balance, after the juice had stood at least an hour.

TITRATABLE ACIDITY.—Because of the high pigmentation of the juices soon after expression, titration by means of an indicator was impossible; hence the electrical conductivity method was employed. The peak of the curve could be read with an error of about ± 0.3 cc. 0.1 N NaOH.

HYDROGEN-ION CONCENTRATION.—The electrometric method was used for determining the P_H of the juices. Considerable trouble was experienced with the poisoning of the electrode by the

juices, but with care in the renewal of the platinum an accuracy of 0.3 millivolt was obtained.

TANNIN.—The Procter-Löwenthal method as detailed in the Official Methods (1) was used.

OXALIC ACID.—In the case of the juice, 40 cc. were treated with 80 cc. of 95 per cent alcohol, filtered, washed, and made up to 200 cc. Aliquots of this were used for oxalic and for malic and tartaric acids. For oxalic the alcohol was evaporated, the material neutralzed with ammonia, acidified with acetic acid, filtered if necessary, treated with calcium acetate in the hot, the calcium oxalate filtered off, and titrated with permanganate. The precipitate no doubt was impure, as it adsorbed some coloring matter, but a satisfactory end point was obtained, and the results are at least comparative. In the case of the residue, 10 gm. was digested with 75 cc. of 0.8 per cent hydrochloric acid for one hour, to liberate any oxalate existing in the residue in the form of the calcium salt. The extract was treated as in the case of the juice.

NITRITES.—The qualitative test with α-naphthylamine and sulfanilic acid was used.

PROTEIN AND NON-PROTEIN NITROGEN.—Trichloracetic acetic acid was used as the protein precipitant. BLISH's (8) copper hydrate method was tried in comparison with the trichloracetic acid. Since the latter gave the same results and is simpler to use, it was adopted. Twenty cc. of juice plus 20 cc. of 25 per cent trichloracetic acid were allowed to stand overnight, and the nitrogen in a filtrate determined. In the case of the residue a weighed sample was extracted with boiling water, and the filtered extract treated with the protein precipitant. It was realized that in the case of the residue two factors for precipitating proteins were used, heat and the trichloracetic acid. This fact might give incomparable results on the two sets of samples, but there was no other apparent way of getting the data on the residue.

MALIC AND TARTARIC ACIDS.—The optical method (51) for obtaining these two acids in the same solution was used, since it was thought possible that both were present, although BIGELOW and DUNBAR (5) found only malic. If only one of the two acids be present when this optical method is used, the rotation readings

should indicate zero for the one absent. In applying the method to the plum samples, the results in general did give zero values for tartaric. In some cases, however, positive values were obtained, and in others even negative values. Since these anomalies indicated the presence of some interfering substance, it was found necessary to reject all the data on these two acids; hence they are not presented here, although it was deemed well to record this failure of the double polarization method on plum juices.

Experimental data

CHARACTERISTICS OF ROT.—In table I are listed the varieties of plums used, the abbreviations for these varieties used throughout this paper, the dates of taking samples, and the length of time required for rotting in each case. Probably the same degree of rotting was not obtained in all cases, but the relative rates of progress of the fungus attacks are no doubt fairly well indicated

TABLE I

SAMPLING DATA FOR VARIETIES OF PLUMS USED

VARIETIES	ABBREVIATIONS USED	STAGE I, HALF GROWN		STAGE II, FULLY GROWN*		STAGE III, RIPE	
		Picked	Days required to rot	Picked	Days required to rot	Picked	Days required to rot
Resistant							
Burbank × Wolf 9.....	B × W9 or 9	July 12	15	Aug. 25	17	Aug. 31	15
Burbank × Wolf 16.....	B × W16 or 16	July 12	13	Aug. 20	18	Aug. 28	15
Abundance × Wolf 30.....	A × W30 or 30	July 12	13	.....	.....	.....	.....
Susceptible							
Compass.....	C	July 12	9	Aug. 3	11	Aug. 20	12
Sand Cherry × Formosa...	SCF	July 12	10	Aug. 3	13	Aug. 11	12

by the figures. It is clear that the varieties listed by the horticulturists as resistant are considerably more slowly rotted than are the susceptible varieties. There is more difference in this respect in the earlier than in the later samples. This is in accordance with the commonly observed characteristics of fruit diseases, that in general they become less resistant as maturity approaches. Because of this, the first set of samples was observed more keenly than the following ones, and some interesting points were noted. In connection with the rate of spread of the rot, the time required for

the surface of the fruits to become brown was recorded, as shown in table II. These figures show the same slower rate of rotting of the resistant varieties.

TABLE II

BEHAVIOR OF VARIOUS PLUM VARIETIES TOWARD ROTTING ORGANISM AT FIRST STAGE OF GROWTH

VARIETIES	DAYS REQUIRED FOR SURFACE TO BECOME BROWN	DAYS REQUIRED FOR COMPLETE ROTTING	RELATIVE ABUNDANCE ON SURFACE OF	
			Hyphae	Spores
Resistant				
Burbank×Wolf 9.....	13	15	++	+
Burbank×Wolf 16.....	7	13	+++	+
Abundance×Wolf 30.....	6	13	+	+++
Susceptible				
Compass.....	5	9	+++	+++
Sand Cherry×Formosa..	5	10	+++	+++

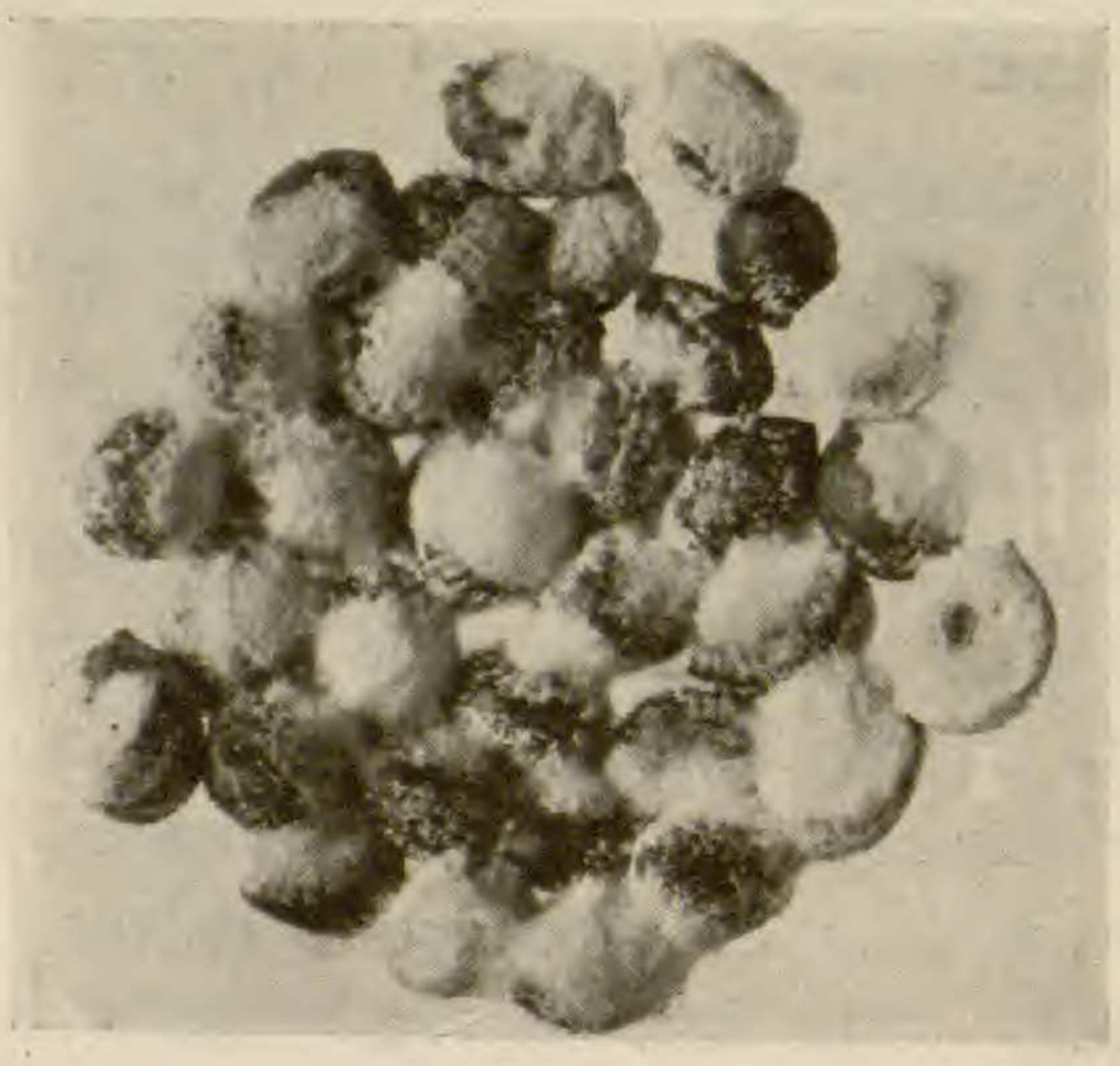

FIG. 1

FIG. 2

FIGS. 1, 2—Fig. 1, dish of Burbank×Wolf 16 plums at end of rotting period in half-grown stage of growth (table II), showing abundance of hyphae on surface and relative scarcity of spore tufts; fig. 2, dish of Abundance×Wolf 30 plums at end of rotting period in half-grown stage of growth (table II), showing abundance of sporulation and scarcity of hyphae on surface.

Another varietal difference was the character of the aerial portions of the fungus, especially as to the relative abundance of spore tufts and of hyphae. These data are recorded in table II. Although the differences among the varieties were well marked, they are not correlated in any striking way with resistance

characters. The susceptible varieties show more abundant aerial growth of both sorts than do the resistant. Possibly the amount of surface growth is dependent on the vigor of the subsurface growth, and the latter is no doubt less in the resistant varieties (figs. 1, 2).

Another varietal characteristic was the relative firmness of the rotted fruit. The sound fruit of the resistant varieties was somewhat more firm than that of the susceptible, particularly in the ripe stage. After rotting, the differences were even more marked. Although this fungus causes what is usually called a firm rot, the rotted fruit of the *C* and *SCF* varieties was almost watery in some instances; while the resistant varieties maintained a firm or even hard texture. This phenomenon may have to do with the character of the pectins, as has been suggested by others. The pectin relations of hosts and parasites offer a fruitful field of investigation (53).

Chemical analyses

Instead of presenting the analytical results in tabular form, which would be rather involved and cumbersome, they are given in the form of charts (figs. 3–7). The data could conceivably be grouped in many different ways, so as to show (1) the comparison of the fresh fruit of the several varieties; (2) the progressive changes during the ripening process; (3) the changes involved during storage in the laboratory, both with and without the action of the fungus; and (4) the effect of the rotting process. This would mean four different groupings of the data in four sets of charts. It was decided to limit this to two groupings. The first set, figs. 3 and 4, bring together side by side the data showing the change in composition of the samples during the storage and rotting in the laboratory. The fresh samples in each case are designated *a*, the sound samples stored in the laboratory without inoculation *b*, the rotted samples *c*. In these charts it is easy to follow the changes brought about by the rotting, and the changes taking place during the three stages of growth, by comparing all the *a* samples, the *b* samples, and the *c* samples in each variety.

The second set, figs. 5–7, bring together side by side the data for comparing the various varieties, that is, the *a* samples for all

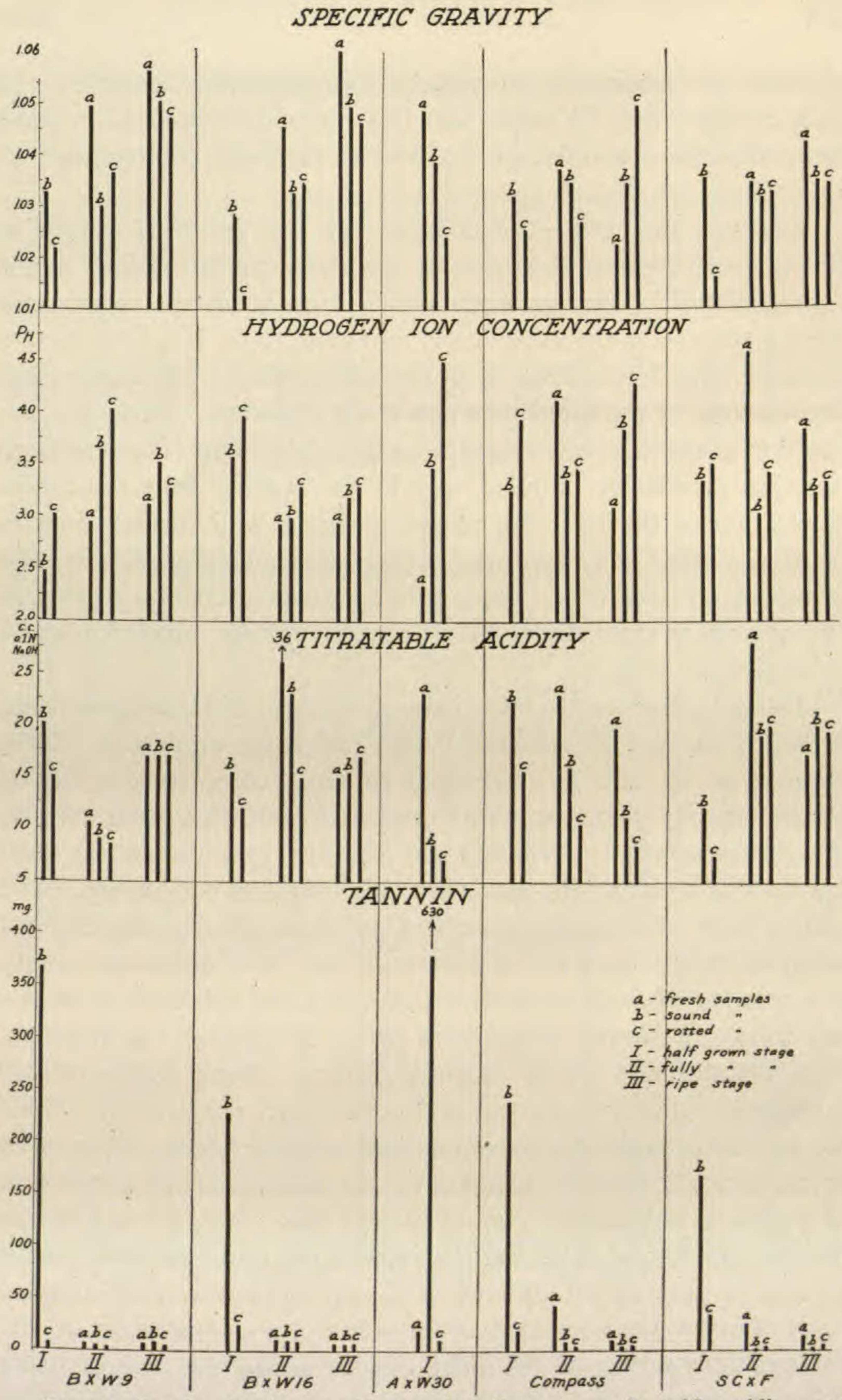

FIG. 3.—Graphs showing specific gravity, P_H values, titratable acidity, and tannin content of juices of all plum samples; figures for titre and tannin are on basis of 10 gm. of juice.

varieties are assembled, then the *b*, and then the *c* samples. In each case the three (in some cases two) resistant varieties are given first, then the non-resistant, in order to facilitate comparison. A brief discussion of each factor will be given.

SPECIFIC GRAVITY.—Referring to the top group of graphs in fig. 3, it will be seen that in most cases the specific gravity of the expressed juice decreases from *a* to *b*, that is, in the sound fruit during storage in the laboratory, and that there is a still further decrease from *b* to *c*, that is, in the rotting fruit. In many cases the decrease in the rotted samples is very marked. Probably respiration consumes sugar in the sound fruit with a consequent decrease in density of juice, and in the rotting fruit the added respiration of the invading fungus causes a still further drop in density. STEVENS and HAWKINS (43) noted a similar phenomenon in rotting strawberries. There is one marked exception to this, in the case of the third stage of the Compass variety, but this may be an analytical error.

In fig. 5 there are indications of varietal differences in juice density that may be correlated with resistance properties. Thus varieties 9, 16, and 30 (resistant) in most cases have a higher specific gravity than varieties *C* and *SCF*, and this holds even in the rotted samples. Whether the osmotic pressure of the host sap may be a controlling factor for this fungus is not known. The writers know of no measurements of its tolerance to strong nutrient solutions except the work of HAWKINS (30), who tested the ability of a number of fungi to grow on concentrated solutions of sugars and salts. *S. cinerea* would grow on 2.4 M glucose, 1.4 M potassium nitrate, and 0.6 M calcium nitrate. These figures would correspond roughly to 43, 14, and 9 per cent, respectively, which are far higher than any concentrations of fruit juices. It is to be noted, however, that HAWKINS gives no information as to the rate of growth at the various concentrations used; hence it is possible that the differences found in the saps of the plum varieties might account in part for the differences in rate of growth of the fungus.

HYDROGEN-ION CONCENTRATION.—In fig. 3 a comparison of the changes in reaction of the juice can be made, the values being given in terms of P_H. No consistent and striking differences are

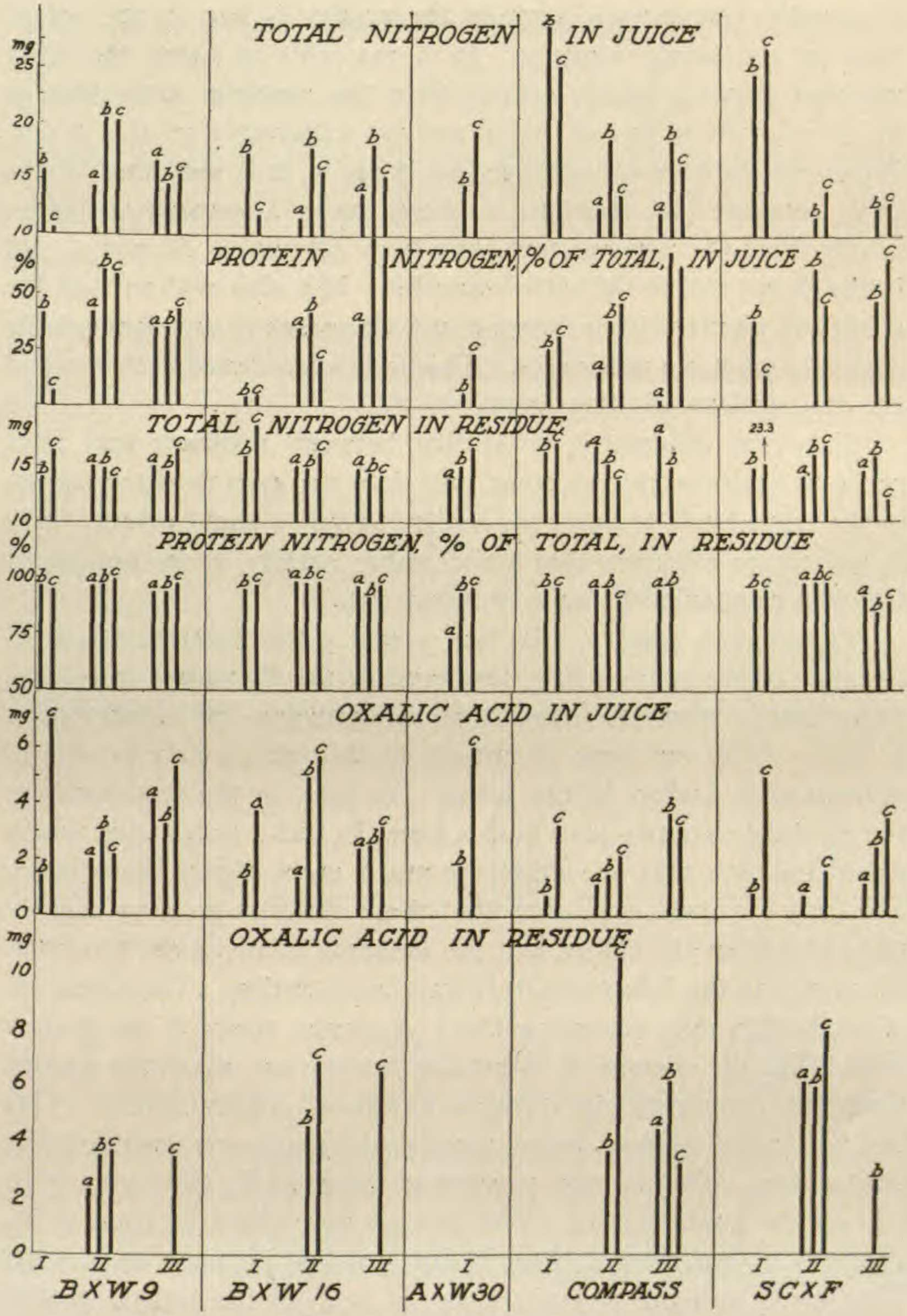

FIG. 4.—Graphs showing total nitrogen, protein nitrogen, and oxalic acid in juices and residues of all plum samples; figures are on basis of 10 gm. of juice and 1 gm. of residue.

discernible, but in most samples the acidity is less in the rotted than in the sound samples. In a majority of cases the fresh material shows a higher acidity than the material after storage in the laboratory, but the data are not conclusive on this point. When the analyses are arranged as in fig. 5, it is seen that in the fresh samples the resistant varieties have a somewhat higher acidity that the non-resistant, but such differences do not obtain in the stored and in the rotted samples. It is also evident that the acidity in plums neither increases nor decreases to any appreciable degree as ripening progresses. This is in accordance with most of the observations on other fruits.

Since the differences in acidity between resistant and non-resistant varieties are not great, and since the growth of the fungus in the tissue tends to lower the acidity to only a slight extent, there is not much evidence that unfavorable acidity is an important factor in resistance of plums to brown rot.

TITRATABLE ACIDITY.—In figs. 3 and 5 the determinations of the titre of the juices follow the trend of the P_H values in reverse order, that is, when the titre is high, the hydrogen-ion concentration is high. There are some exceptions to this, which may be due to differences in buffers in the juices. In fact, in the fresh samples the resistant varieties have both a lower P_H and a lower titre, which would indicate that the acids are much more highly dissociated. The data for oxalic acid show that these varieties do have slightly more of it than the others, but the amounts involved are too small to amount to the differences in H-ion concentration. The character of the buffers may determine this. A careful study of the graphs shows that the changes in titratable acidity are relatively greater than the corresponding changes in H-ion concentration. This becomes more evident when numerical values are used for the comparison. The average percentage decrease in titre from *b* to *c* in all the samples is 17. The average percentage increase in P_H values is 9 (assuming a theoretically possible increase to $P_H = 7$). This would indicate a consumption of acid by the fungus, rather than a production of buffer, in modifying the reaction during rotting. It was hoped that the malic acid determinations would give direct evidence on this point. These determinations will be

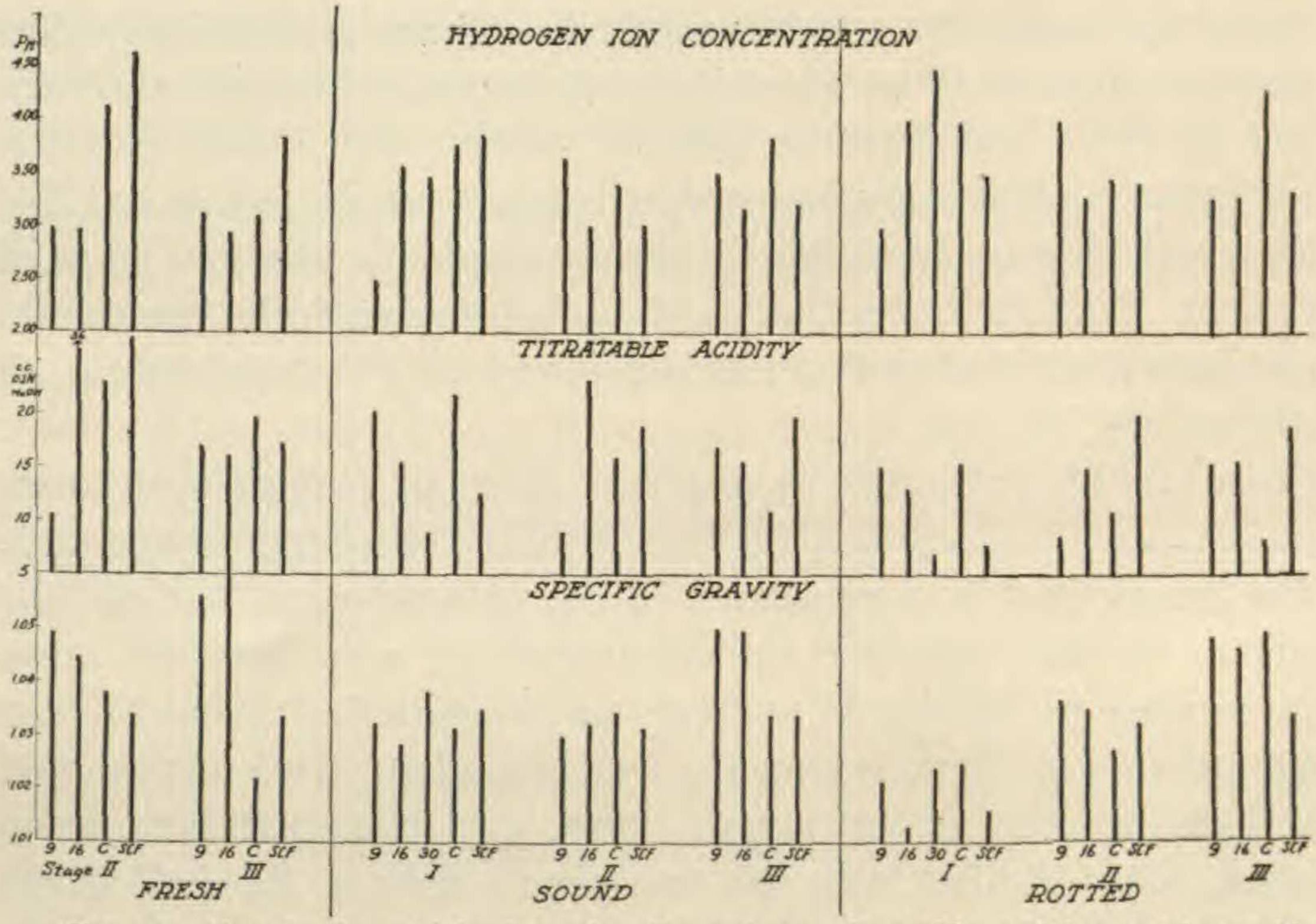

FIG. 5.—Graphs in which data for P_H values, titratable acidity, and specific gravity are assembled to bring together resistant and non-resistant varieties of plums for direct comparison; varieties 9, 16, and 30 are resistant (see table I and fig. 3).

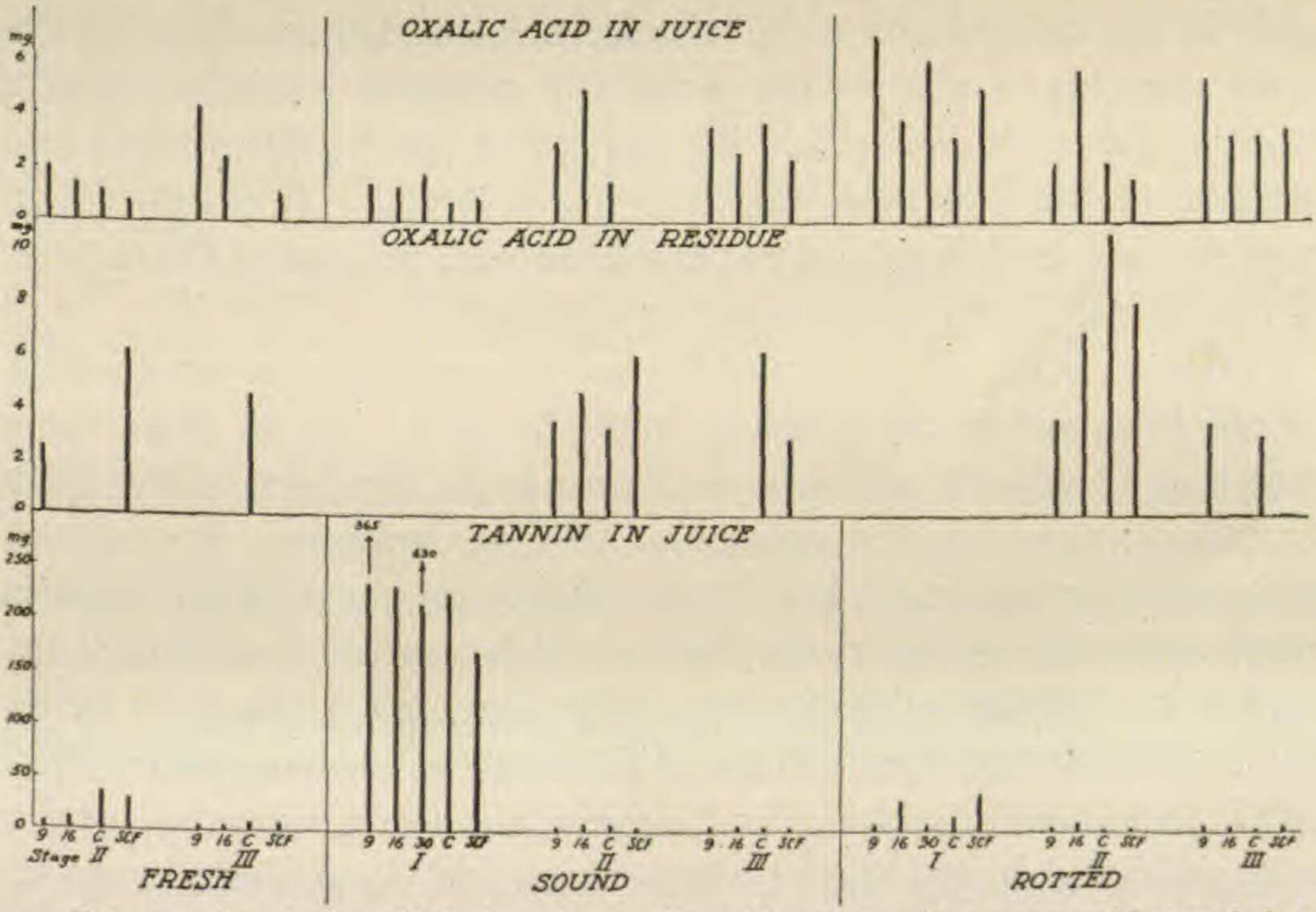

FIG. 6.—Graphs in which data for oxalic acid and tannin are assembled to bring together resistant and non-resistant varieties of plums for direct comparison (see table I and figs. 3, 4).

attempted again by another method. Citrus mottle leaf offers another example of an interesting discrepancy between the titre and the H-ion concentration (36).

TANNIN.—The conspicuous fact brought out in figs. 3 and 6 is the great increase in tannin in the *b* samples in the first stage of growth. This indicates that in these half-grown fruits the tannin increases rapidly after the fruit is picked from the tree, but that if the fruit is infected by the fungus the tannin does not increase. COOK and his colleagues (3, 16, 17) report an enzyme that forms tannin rapidly either when the fruit is picked or when it is wounded. The former fact is corroborated in the present work, but not the latter. In fact, infection by the fungus not only does not cause an increase in the tannin content, but in varieties *C* and *SCF* in fig. 3 there is a decrease over the fresh samples. Two facts should be kept in mind in this regard: first, that the great increase in tannin after picking from the tree occurs only in the half-grown stage of growth; and second, that the decrease in tannin in the rotted samples is noticeable only in the two later stages of growth, since unfortunately in the first stage the fresh sample was analyzed only in the case of $A \times W30$. In fig. 6 it can be seen that in the fresh samples of the second stage the resistant varieties have a lower content of tannin than the susceptible, and that in the sound samples of the first stage the facts are reversed. It is difficult to perceive any facts that can be correlated with resistance characters. VALLEAU (46) came to the same conclusion.

OXALIC ACID.—Figs. 4 and 6 give the analyses for oxalic acid in the juice and in the residues from the juice. In all cases there was a small amount of oxalic acid present in the juice of the fresh fruit, as judged by the reduction of permanganate. It does not average over 0.02 per cent of the juice. In most cases there is more oxalic acid in the *c* than in the *a* or *b* samples, indicating that during the rotting a production of the acid takes place. This is in accordance with the findings of COOLEY (19), who reported that oxalic acid was produced by *Sclerotinia*. The amount of oxalic acid produced in the rotted plums, however, seems insufficient to exert any very marked solvent power on the tissues. Although the data for oxalic acid in the residues are very incomplete, they indicate

the same general trend as do those for the corresponding juices. There are some indications in fig. 6 that the resistant varieties have a higher oxalic acid content than the susceptible, both with and without fungus action in the tissues. If this is found to be the case in future analyses, it may constitute some new evidence on the question of resistance properties.

NITROGEN DISTRIBUTION.—In figs. 4 and 7 are presented the results of the analyses for total and for protein and non-protein nitrogen. There is great irregularity in the quantities of total nitrogen in the juice in the three groups of samples, so much so

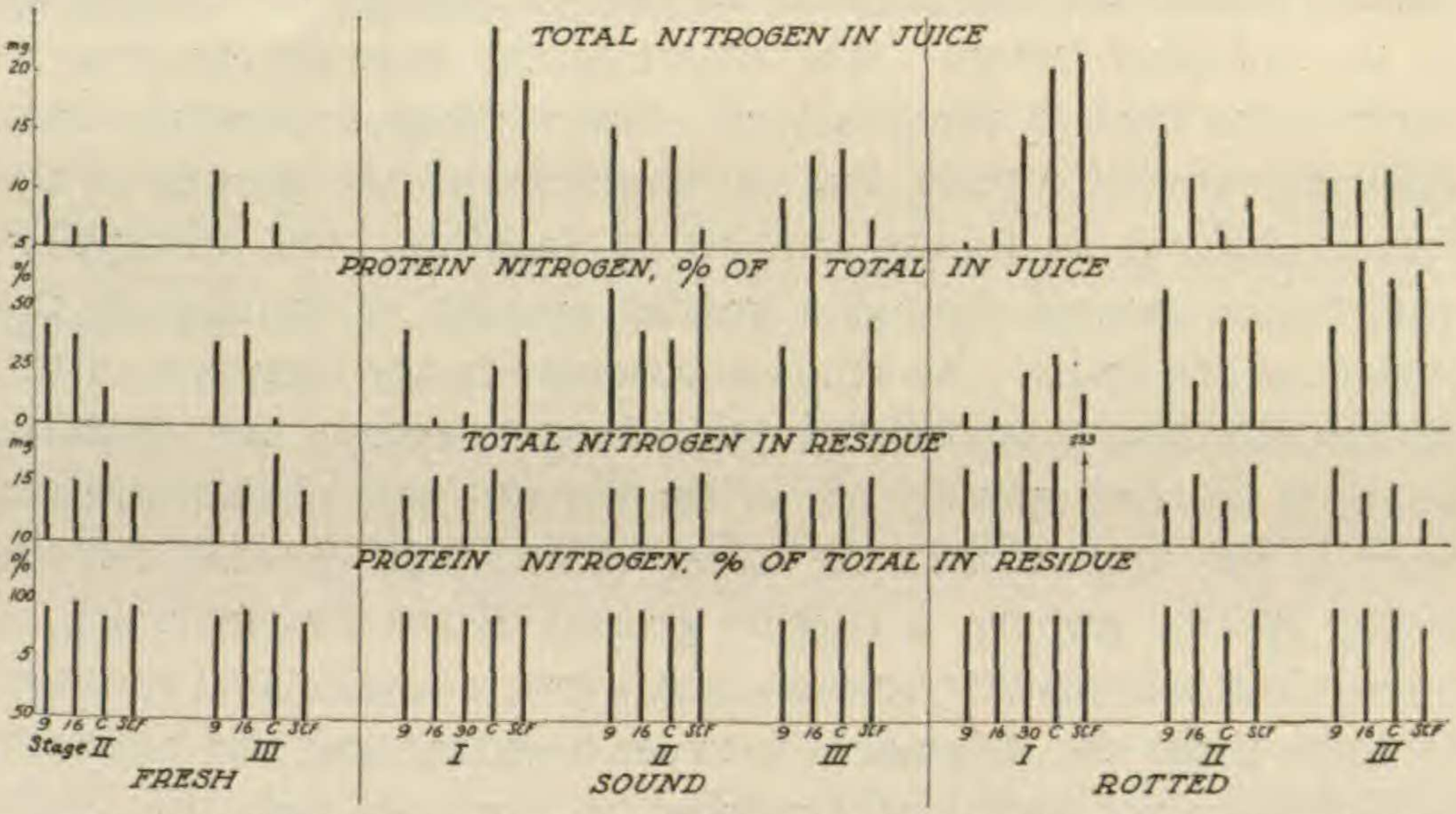

FIG. 7.—Graphs in which data for total and protein nitrogen are assembled to bring together resistant and non-resistant varieties of plums for direct comparison (see table I and fig. 4).

that it is difficult to see any definite trend to the graphs in fig. 4. In the case of the residue, there is some evidence that the rotted samples (*c*) have a greater amount of total nitrogen than the sound (*b*). This is no doubt due to the facts that a far greater proportion of the nitrogen is in protein form in the residue, and that the building of fungus protein makes this protein nitrogen still higher in the residues of the rotted samples. No definite trend nor significance can be seen in the data for the protein nitrogen in the juice.

NITRITES.—No test for nitrites was obtainable in any of the samples. The disturbance of the nitrogen nutrition of the host

cannot be a factor in this disease as it seems to be in others (**10, 11, 33, 34**). No varietal differences in the nitrogen content and forms of nitrogen are discernible in fig. 7.

Discussion and summary

The laboratory inoculations recorded in this paper corroborate the field observations on plum varieties as to their relative resistance to the brown rot fungus, *Sclerotinia cinerea*. In the field an important factor in resistance is the thickness of the skin. In the present studies this was eliminated by injecting the spores into the tissues, so that the differences in the rate of rotting were due mostly to physiological factors. The object of the investigation was to throw some light on these factors. The varieties showed not only different rates of rotting, but the character of the growth of the fungus differed as to the amount of fruiting. The susceptible varieties in general showed a greater amount of fruiting on the surface of the fruits. No study is recorded in the literature of the factors affecting sporulation in this fungus, except the vitamine relations touched upon by one of the writers (**52**). In the present work it was noticed that the juices of resistant varieties have a higher specific gravity, a slightly greater H-ion concentration, a lower titratable acidity, and a slightly greater oxalic acid content. In these items the differences between resistant and non-resistant varieties are not sufficiently marked to convince one that they constitute the chemical basis of resistance. Culture work with *Sclerotinia*, using fruit juices in which the various factors can be varied and controlled, will no doubt throw considerable light on the question.

When the fungus rots the plum, some well marked changes in composition take place in the tissues. The juices show considerable decrease in specific gravity, a decrease in true acidity, a decrease in titratable acidity that is of greater magnitude than the decrease in true acidity, and an increase in oxalic acid content. The fungus in some way prevents the production of tannin that takes place in the green fruit after it is picked from the tree. The fungus converts a portion of the non-protein nitrogen of the host into protein nitrogen in its own mycelium.

Nitrites could not be detected in any of the samples. They are probably not a product of the rotting by this fungus. No hypothesis can be suggested as yet for the chemical and physiological basis of resistance in the brown rot of stone fruits.

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